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Original Paper

Monoclonal Antibody to Human Trk-A: Diagnostic and Therapeutic Potential in Neuroblastoma

K. Kramer,[1] W. Gerald,[2] L. LeSauteur,[3] H.U. Saragovi[3,4] and N.-K.V. Cheung[1]

[1]Department of Pediatrics; [2]Department of Pathology, Memorial Sloan-Kettering Cancer Center, New York, New York 10021, U.S.A.; [3]Department of Pharmacology and Therapeutics; and [4]McGill Cancer Center, McGill University, Montreal, Quebec H3G 1Y6 Canada

5C3 is a murine IgG_1 antibody specific for the nerve growth factor (NGF) docking site of the human p140 trk-A receptor, with no cross-reactivity with human trk-B. *In vitro*, 5C3 and its Fab mimic the effects of NGF, a neurotrophin mediating growth and differentiation of neural crest-derived cells. When labelled with radioisotope, 5C3 images human trk-A positive tumours *in vivo*. More importantly, 5C3 induces regression of human trk-A positive tumours in rodents. We therefore investigated the value of 5C3 in detecting trk-A expression in human neuroblastoma by immunohistochemistry. 5C3 reactivity was detected in 73 of 113 neuroblastoma specimens and correlated strongly with localised/4s disease (55/60) with either a homogeneous or mixed pattern. Among stage 4 neuroblastoma, only 18/53 had homogeneous or mixed trk-A expression. 5C3 did not react with 46/48 other human malignancies, but was positive in 1 melanoma and 1 Wilms' tumour specimen. The prognostic, imaging and NGF-mimetic properties of antibody 5C3 and its derivatives may offer alternatives for the diagnosis and treatment of neuroblastoma. © **1997 Elsevier Science Ltd.**

Key words: trk-A, p140 trk-A nerve growth factor receptor, neuroblastoma, paediatric malignancies
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INTRODUCTION

NERVE GROWTH factor (NGF) is a member of the neurotrophin family and mediates growth and differentiation of neural crest-derived cells [1, 2] The effects of NGF are mediated by the receptor trk-A. Trk-A is expressed in many primary neuroblastomas and trk-A mRNA expression has been associated with favourable outcome in human neuroblastoma [3, 4]. A mouse monoclonal antibody (MAb), 5C3, generated against the extracellular domain of human p140 trk-A, competes with NGF in trk-A binding assays to human trk-A-expressing E25 cells [5]. 5C3 mimics NGF function in receptor phosphorylation, receptor internalisation, protection of apoptotic death in serum-free media and increased transformation of trk-A-expressing cells [5]. 5C3 is specific for human p140 trk-A and has demonstrated no cross-reactivity with other neurotrophin receptors such as human trk-B or p75 [5]. Preclinical studies in mice bearing human trk-A-positive tumours have demonstrated the imaging potential of radiolabelled 5C3 [6]. Trk-A-positive tumour engraftment in rodents was inhibited by 5C3 given by intraperitoneal injection; primary tumour growth was significantly inhibited while metastases were prevented [7]. We therefore used MAb 5C3 to detect trk-A expression in human neuroblastoma and to correlate trk-A protein expression with stage and clinical outcome.

Correspondence to N.-K.V. Cheung.

MATERIALS AND METHODS

Frozen tumours from 113 patients with neuroblastoma and 48 tumours from patients with other malignant tumours were analysed. Patients with neuroblastoma were stratified according to stage by the International Neuroblastoma Staging System. Sixty patients had localised/stage 4s disease (group 1) and 53 had metastatic stage 4 disease (group 2). Trk-A protein expression was analysed by immunohistochemistry using MAb 5C3 and the avidin–biotin peroxidase complex (Vector Laboratories, Burlington, California, U.S.A.) and a 10% haematoxylin counterstain. Sections were graded as positive (>90% of cells immunoreactive), mixed (10–90% of cells immunoreactive) or negative (<10% of cells immunoreactive).

Table 1. *Trk-A detection using MAb* 5C3 *by immunohistochemistry in human neuroblastoma*

Neuroblastomas	*n*	Positive (%)	Mixed (%)	Negative (%)
Group 1	60	38 (64)	17 (28)	5 (8)
Group 2	53	13 (25)	5 (9)	35 (66)
Samples at diagnosis	26	5 (19)	4 (16)	17 (65)
Infants less than 1 year of age	10	2 (20)	1 (10)	7 (70)

RESULTS

5C3 detected p140 trk-A expression in 73 (65%) of the 113 neuroblastoma tumours. In general, the small round, blue cells typical of undifferentiated neuroblastoma had minimal or no staining for trk-A and staining was most prominent in the mature ganglion cells and their cellular processes.

Trk-A protein expression strongly correlated with localised/4s neuroblastoma ($P < 0.0001$). A high level of homogeneous or mixed immunoreactivity was found in 55 of 60 (92%) tumours of group 1 patients, while only 18 of 53 (34%) tumours in patients group 2 demonstrated similar reactivity. Lack of trk-A protein expression strongly correlated with stage 4 neuroblastoma. Infants less than 1 year of age at the time of diagnosis demonstrated a near identical pattern of trk-A expression as older patients (Table 1). No 5C3 reactivity was found in breast carcinomas, lung carcinoma, glioma, medulloblastoma, desmoplastic small round cell tumour, Ewing's sarcoma, PNET, osteosarcoma or rhabdomyosarcoma. One of 9 melanoma samples and 1 of 6 Wilms' tumours were noted to have 5C3 reactivity.

DISCUSSION

NGF's interaction with trk-A appears to play a role in human neuroblastoma cell growth, differentiation and cell death. Recent data supports the role of other neurotrophins such as pleiotrophin and trk-B in the regulation of neuroblastoma tumorigenesis and invasiveness [7–9]. Using MAb 5C3, we detected human p140 trk-A expression in neuroblastoma. Expression correlated with localised/4s disease, a clinical group characterised by a favourable outcome. Lack of trk-A protein expression was correlated with metastatic malignant neuroblastoma. A clear survival advantage was seen among patients with 5C3 immunoreactivity, not an unexpected finding given the close correlation of trk-A expression with stage. A subset of stage 4 patients, however, still demonstrate trk-A immunohistochemical expression, implying that other biological parameters dictate the clinical behaviour of neuroblastoma in these cases. Among small, round blue cell tumours, 5C3 immunoreactivity was specific for neuroblastoma.

We conclude that 5C3 is a useful tool to detect p140 trk-A expression by immunohistochemistry. Trk-A protein expression using 5C3 identifies patients more likely to have localised/4s neuroblastoma. As a mimic of NGF function, 5C3 or receptor binding small peptide mimetics [10] may be useful in the radioimaging and treatment of trk-A expressing tumours.

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